Prevention of Lens Damage Associated with Cigarette Smoke Exposure in Rats by α-Tocopherol (Vitamin E) Treatment

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PURPOSE. To evaluate the possible protective effect and mechanism of α-tocopherol (vitamin E) treatment on lens degeneration associated with in vivo exposure to cigarette smoke and to further clarify the role of iron in cigarette smoke-generated lens damage.

METHODS. Twenty-eight male Wistar rats were randomly divided into four equal groups. Rats in groups 3 and 4 were exposed to cigarette smoke for 1 hour each day over 90 consecutive days, and rats in groups 1 and 2 were treated in similar fashion but only exposed to room air. Additionally, vitamin E was given to the rats in groups 2 and 4 via intramuscular route. At the end of the study, both eyes of all the animals were enucleated; one eye was prepared for histopathologic examination, and the fellow eye was used for the measurement of iron and calcium levels.

RESULTS. Significantly higher iron and calcium levels were observed in the lenses of group 3 rats than in other groups. Similar comparisons performed between groups 1 and 2, groups 1 and 4, and groups 2 and 4 did not show any significant difference. Distinct histopathologic changes in the anterior lens epithelium, such as hyperplasia, hypertrophy, epithelial multilayering, and the presence of epithelial cells over posterior lens capsule, observed in group 3 rats were not present in other groups.

CONCLUSIONS. Cataractogenesis after cigarette smoke exposure was associated with an accumulation of iron and calcium in the rat lens, and vitamin E supplementation protected such accumulations and cataractogenesis. (Invest Ophthalmol Vis Sci. 1999;40:537-541)

The relationship between cigarette smoking and human cataract formation was depicted in epidemiologic studies. The most possible mechanism by which cigarette smoke contributes to cataractogenesis is oxidative damage, because cigarette smoke-exposed tissues contain large amounts of reactive oxygen species (ROS) and metals. Accumulation of ROS in the eye lens may contribute to cataractogenesis. ROS in living tissues can be generated via two routes. ROS can be produced photodynamically, through the mediation of sensitizing molecules that have been excited to higher electronic states by light absorption, and also through the Fenton reaction. Significant lenticular damage and generation of ROS have been shown in the smoke-exposed rat lenses even in the absence of light. Thus, oxygen free radicals in the smoke-exposed rat lenses most probably occurs via Fenton metal-catalyzed reactions. Various metals, which can undergo univalent redox reactions, can participate in the enzymatic and nonenzymatic oxidation and peroxidation of biological molecules reducing O2 to more toxic oxygen free radicals, H2O2 and OH−, using the Fenton reaction. Of these metals, manganese, iron, cobalt, and copper are of biologic importance, but iron is the most effective catalyst in these oxidative processes.

We have recently reported that iron concentration in rat lenses was increased after smoke exposure and that this increase was associated with histopathologic evidence of lens damage and a decrease in lens zinc concentration. The decrease in lens zinc concentration was accepted as indirect evidence of oxidative lens damage. The present study was planned to analyze the possible protective effect of antioxidant-tocopherol (vitamin E) in rat lens damage after cigarette smoke exposure and to gain a better understanding of the oxidative nature of cigarette smoke-associated damage in the rat lens.

MATERIALS AND METHODS

Twenty-eight male Wistar rats (200-250 g body weight, 10-12 weeks old) were randomly divided into four equal groups. The rats were housed in stainless-steel wire cages except during smoke exposure and fed with standard rat chow and tap water ad libitum. The rats used in this study were kept from any distress during the study period. The study was carried out according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Group 1 (mean ± SD; age, 205 ± 5 weeks) was exposed to cigarette smoke for 1 hour each day over 90 consecutive days, and rats in groups 1 and 2 were treated in similar fashion but only exposed to room air. Additionally, vitamin E was given to the rats in groups 2 and 4 via intramuscular route. At the end of the study, both eyes of all the animals were enucleated; one eye was prepared for histopathologic examination, and the fellow eye was used for the measurement of iron and calcium levels.

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paraformaldehyde. The lens samples were prepared with autopsie. The anterior portions of both eyes of each rat was rolled in filter paper to remove all adherent vitreous and stained with hematoxylin-eosin. The specimens were ob-
given to groups 2 and 4 nits via the intramuscular route in a vitamin E-treated rats. The age of the rats did not differ statis-
tically between groups. “Maltepe” cigarettes (TEKEL Cigarette Factory, Yistanbul, Turkey) manufactured from a blend of Turkish tobacco were used in the study. Groups 3 and 4 rats were exposed to cigarette smoke for 1 hour each day over 90 consecutive days using a smoking machine. Groups 1 and 2 rats were restrained in identical chambers but only exposed to room air. Immediately before either smoke or sham exposure, vitamin E (synthetic all-rac tocopherol, prepared from synthetic phytol, C29H50O2 Sigma catalog No. 1997/T 3251; Sigma Aldrich Chemie GmbH, Diesenhofen, Germany) was given to groups 2 and 4 rats via the intramuscular route in a dose of 10 mg/kg over 90 consecutive days.

Smoke Generation and Exposure
The smoke exposure system consists of three glass chambers, fans, and pumps. A detailed description of the system can be found in an article previously published by our group.

Preparation of the Tissue Samples and Determination of the Metal Levels
Both eyes of all rats were enucleated under high ether anes-
thesia after the last exposure (no later than 2 hours after the last exposure) and kept without cooling in an insulated con-
tainer until lens dissection performed no later than 2 hours postmortem. The anterior portions of both eyes of each rat were removed by cutting just posterior to the limbus under the magnification of a coaxial operating microscope and stainless-steel surgical equipment. The lens was removed after the suspensory ligaments were carefully cut, taking great care to avoid contamination from neighboring tissues and environ-
mental sources. One of the freshly dissected lens of each rat was rolled in filter paper to remove all adherent vitreous and iris and then dried at 80°C for 24 hours. The dried lens was weighted to the nearest 0.1 mg using Sartorius Basic Electrobal-
ance (model BA 1105; Göttingen, Germany) and transferred into a glass vial prewashed with trace element-free HNO3. After cleaning the lens with deionized water, it was digested by heating at 140°C in a mixture of concentrated nitric and perchloric acids (in a volume ratio of 5/1) until the organic matrix was completely dissolved. The mouth of the tube was covered with paraffin and stored at 4°C until analysis could be performed. Calcium and iron concentrations were determined by flame atomic absorption technique (Spectra AA Varian, model 400; Kirkbright, Sydney, Australia). The element content was expressed as micrometers per gram of dry tissue weight. All measurements were performed on a masked basis.

The fellow lens of each rat was fixed in 10% buffered paraformaldehyde. The lens samples were prepared with auto-
techincon and then embedded in paraffin. Six slices (5-μm thick) were obtained for each lens with a microtome and stained with hematoxylin-cosin. The specimens were ob-
erved and photographed using a photomicroscope (Carl Zeiss, Oberkochen, Germany). The specimens were read on a masked basis. A total of 42 lens specimens were analyzed for each group.

### RESULTS

The mean iron and calcium concentrations in the lenses of the group 1 rats were 6.76 μg/g and 3.17 μg/g (dry tissue weight), respectively. In the group 2 rats, mean iron concentration was measured as 6.71 μg/g and calcium concentration as 3.34 μg/g. On the other hand, mean iron and calcium concentrations in the group 3 rats were measured as 16.84 μg/g and 9.94 μg/g, respectively. Mean iron concentration in the lenses of group 4 rats was 8.33 μg/g, and mean calcium concentration was 3.77 μg/g. Statistical analyses revealed significantly higher lens iron and calcium levels in group 3 rats than in the other groups. Similar comparisons performed between groups 1 and 2, groups 1 and 4, and groups 2 and 4 did not reveal any significant difference (Table 1).

Anterior lens epithelial cells were unilayered and com-
posed of low cuboidal–cylindric type of epithelium in rats of groups 1, 2, and 4, and no epithelial cells were observed overlying the lens capsule behind the equator of the lens. Epithelial cells had a fusiform and scanty cytoplasm with an oval-shaped nucleus in all the analyzed specimens belonging to groups 1 and 2 (Fig. 1). However, the arrangement of lens epithelial cells of group 3 animals showed entirely different features than the control group. Multilayering of anterior ep-
ithelial cells was present in all slices. The cytoplasm lost its fusiform appearance and became more abundant, and the nuclei became larger and more spherical in relation to the anterior epithelia of groups 1 and 2 (Fig. 2). Furthermore, we observed edematous epithelial cells over the posterior lens capsule in all slices belonging to group 3 animals (Fig. 3). However, slices from group 4 animals contained a different type of epithelium. Although the anterior lenticular epithelium was unilayered in all slices, the nuclei of the cells were more spherical compared with groups 1 and 2 (Fig. 4). However, any epithelial cells over the posterior lens surface did not present in any histopathologic slice belonging to the group 4 rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron</th>
<th>Calcium</th>
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<tbody>
<tr>
<td>1 (sham-exposed rats)</td>
<td>6.75 ± 3.90</td>
<td>3.17 ± 0.65</td>
</tr>
<tr>
<td>2 (sham-exposed + vitamin E-treated rats)</td>
<td>6.71 ± 4.07</td>
<td>3.34 ± 0.96</td>
</tr>
<tr>
<td>3 (smoke-exposed rats)</td>
<td>16.84 ± 2.88</td>
<td>9.94 ± 3.29</td>
</tr>
<tr>
<td>4 (smoke-exposed + vitamin E-treated rats)</td>
<td>8.33 ± 4.20</td>
<td>3.77 ± 0.56</td>
</tr>
</tbody>
</table>

Values are means ± SD, expressed as μg/g dry tissue weight. All groups contained 7 male Wistar rats. Statistical comparisons performed between groups 1 and 2, 1 and 4, and 2 and 4 did not reveal any significant difference in either iron or calcium levels. However, similar comparisons performed between groups 1 and 3, 2 and 3, and 3 and 4 showed statistically significant differences (all *P* < 0.05). Statistical analyses were performed by using Kruskal-Wallis one-way ANOVA.

### Statistical Analysis

The results were expressed as means ± SD. Kruskal-Wallis one-way ANOVA was used for statistical comparisons. Statistical significance was accepted as *P* < 0.05.
FIGURE 1. A specimen from the central region of group 1 rat lens (sham-exposed group). The anterior lenticular epithelium (A) is unlayered and composed of low cuboidal-cylindric type cells. Epithelial cells are observed as having fusiform cytoplasm and oval nucleus. The lens specimens belonging to group 2 rats (sham-exposed group) share similar characteristics (hematoxylin-eosin, ×40).

DISCUSSION

We recently reported increased iron and calcium levels and decreased zinc concentrations in lenses of cigarette smoke-exposed rats. This change in the lens metal content was associated with distinct morphologic changes of the anterior lenticular epithelium such as hyperplasia, hypertrophy, and multilayering. Similar changes have been observed in cultured rat lenses after exposure to wood, and cigarette smoke condensates were reported by Shalini et al. and Rao et al. and antioxidants were seen to offer partial inhibition against lens damage. This observation indicated oxidative damage to be a cause of cigarette smoke-induced cataractogenesis. In the present study we have observed a good deal of morpho-

FIGURE 2. A central lens specimen from group 3 (smoke-exposed group). Multilayering of the anterior epithelial cells (A) is the most prominent finding. Also, the cytoplasm and nucleus of the cells are larger and more spherical than those of group 1 lenses (hematoxylin-eosin, ×40).
logic protection against the cataractogenic effects of cigarette smoke exposure in vitamin E-supplemented rats. Vitamin E-treated rats did not develop multilayered epithelium as the smoke-exposed group did and showed only minimal morphologic changes compared with the sham-exposed group.

Histopathologic changes observed in the present study after cigarette smoke exposure (group 3 rats) may indicate cataractous changes, because proliferation of the anterior lenticular epithelium and multilayering are the characteristic histopathologic findings of anterior subcapsular cataracts (e.g., after

**Figure 3.** Posterior central epithelium of the lens specimen belonging to group 3 (smoke-exposed group; B). Note the presence of the nucleated epithelial cells over the posterior lens capsule. This feature is diagnostic for the presence of the cataract. These cells are edematous in appearance (Wedl cells or bladder cells) as a result of imbibition of the proteinaceous fluid derived from liquefied cortical fibers (arrows; hematoxylin-eosin, X40).

**Figure 4.** A lens specimen obtained from central area belonging to an animal in group 4 (smoke-exposed and vitamin E-treated). Although the nuclei of the anterior epithelial cells (A) are spherical-to-oval shaped relative to group 1 (sham-exposed group) animals (arrowheads), epithelial multilayering is no longer observed (hematoxylin-eosin, X40).
inflammation, trauma, or atopic dermatitis). This type of cataract is a common response to many types of irritation. Moreover, there were epithelial cells present over the posterior lens capsule in all slices from group 3 rats. The presence of lens epithelial cells, either normal in appearance or edematous, reflects a posterior migration of lens epithelium from the equator and is diagnostic of cataract. In addition to this migration, the epithelial cells on the posterior lens surface were observed as swollen cells. This is another typical histopathologic finding in cataractous lens. We did not observe these cells in the lenses of vitamin E-treated and smoke-exposed animals. Thus, it can be said that cataractous changes in cigarette smoke-exposed rats can be prevented by vitamin E treatment.

The mechanism of the protective effect of vitamin E therapy on lens damage after cigarette smoke exposure seems to be related to aversion of iron accumulation in the lens tissue. Although the lens iron and calcium concentrations of vitamin E-treated rats were significantly lower than those of the smoke-exposed group, they were not significantly different from those of the sham-exposed rats. It is an important finding, because tissue injury caused by ROS often depends on the availability of iron. Iron can reduce O2 to more toxic ROS by Fenton reaction. Indeed, cigarette tar and tobacco leaves contain large quantities of iron. There is strong evidence that ROS has a role in the pathogenesis of cataract.

The protective effect of vitamin E against smoke-induced cataractogenesis was clearly apparent in the present study. However, the mechanism of this protective effect could not be exactly explained from our results. A possible explanation is that vitamin E therapy of cigarette smoke-exposed rats may prevent the iron influx through the lens capsule and that this effect was associated with the amelioration of morphologic evidence of lens damage. Vitamin E prevents oxidative damage by blocking a defect in electrolyte transport systems in cell membranes induced by ROS. Although previous studies have indicated that vitamin E content of the lens does not increase with supplementation, such supplementation decreases the aqueous humor level of reduced glutathione and lipid peroxides, which are good indicators of oxidative damage. and simultaneously instilled vitamin E-containing liposomes can delay cataractogenesis in young adult rats fed a 25% galactose diet. It is probable that vitamin E can operate on the aqueous side of the lens membrane, but it is clear that this theory could not be proven on the basis of our presented data.

Vitamin E therapy seemed to avert the elevation of the calcium concentration after exposure to cigarette smoke. Increased calcium concentrations observed in group 3 rats provide an additional clue as to lens injury from in vivo exposure to cigarette smoke, because an elevation of cytosolic calcium concentration after exposure to cigarette smoke. In addition to this migration, the epithelial cells on the posterior lens surface were observed as swollen cells. This is another typical histopathologic finding in cataractous lens. We did not observe these cells in the lenses of vitamin E-treated and smoke-exposed animals. Thus, it can be said that cataractous changes in cigarette smoke-exposed rats can be prevented by vitamin E treatment.

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In the current experiment, we used only male rats. Some may think that this fact inevitably leads to a conclusion that our results should be restricted to male rats. However, prevalence studies performed on humans showed that cigarette smoking enhances the cataract incidence in male and female smokers. Also, vitamin E provides protection against cataractogenesis in men and women, but some reservation should be undertaken before applying our results to female rats. Clearly, the two possible routes by which the cigarette smoke reaches the eye lens are (1) through the cornea and (2) the systemic circulation; however, the dominant route is not known.

Our results demonstrate that iron accumulates in rat lens after in vivo exposure to cigarette smoke and that concurrent vitamin E therapy offers some protection against iron accumulation and smoke-induced lens damage. Clearly, additional studies are needed to investigate the mechanism of effects of cigarette smoke exposure on the eye lens and the protective effects of antioxidant vitamins, because a recent long-term study showed no protective effect of vitamin E supplementation for cataract formation in male smokers. Our study provides some clues for the oxidative stress theory and the potential protective role of vitamin E therapy.

References